UNIVERSITI PUTRA MALAYSIA

SACCHARIFICATION OF PALM OIL MILL EFFLUENT SOLID AND OIL PALM FRUIT FIBER TO FERMENTABLE SUGARS FOR ACETONE-BUTANOL-ETHANOL FERMENTATION

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By

KHAW TEIK SEONG

Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Science in the Faculty of Food Science and Biotechnology Universiti Putra Malaysia

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Chairman : **Associate Professor Dr. Arbakariya Ariff**

Faculty : **Food Science and Biotechnology**

The effect of chemical pretreatments on saccharification of palm oil mill effluent (POME) solid and oil palm fruit fiber (OPFF) was investigated. Among the chemical pretreatments applied to the substrate (NaOH 0.5%, NH_3 0.5%, HCl 0.5%, HNO_3 0.5% and EDTA 0.5%), the OPFF treated with 0.5% NaOH gave the highest production of fermentable sugars. However, the saccharification performance for chemically treated POME solid was not significantly different as compared to untreated POME solid. The used of autoclaved OPFF at 121 ^{0}C, 15 psi with NaOH for 5 minute, increased the degree of hydrolysis up to 46% as compared to untreated OPFF. The optimum concentration of NaOH for the treatment of OPFF was 2%. The improvement in hydrolysis of OPFF was related to an increase of cellulose content, and a decrease in hemicellulose and lignin content.

The effect of enzyme and initial substrate concentration on the saccharification of POME solid and OPFF was investigated using two types of cellulolytic enzymes, celluclast 1.5L (47.4 U/mL FPase, 66.0 U/mL CMCase and 51.1 U/mL β-glucosidase) and Novozyme 188 (2.79 U/mL FPase, 10.0 U/mL CMCase and 168 U/mL β-glucosidase). The highest production of reducing sugars (9.24 g/L) and glucose (4.54 g/L) from the saccharification of 5% POME solid was obtained using the Novozyme/Celluclast (N/C) ratio of 0.4. The saccharification of OPEFB using the N/C ratio of 0.25 produced 32.47 g/L total reducing sugar and 16.78 g/L glucose. The effect of initial substrate concentration on the performance of saccharification of POME solid (2% - 20% w/v) and OPFF (2% - 6%) was carried out in 2 liter stirred tank bioreactor. The highest reducing sugar (12.25 g/L) and glucose (6.70 g/l) was obtained when 15% (w/v) POME solid was used. On the other hand, the highest total reducing sugar (30.26 g/L) and glucose (16.73 g/L) was produced from 5% (w/v) OPFF.

The effect of mixing on the performance of the saccharification of CMC, POME solid and OPFF was also carried out in 2 liter stirred tank bioreactor using two different impeller diameters (48 and 84 mm). In saccharification of POME solid and OPFF, the degree of saccharification increased with increasing impeller tip speed (ITP). However, the saccharification of CMC, a soluble cellulose, increased with increasing ITP up to 2.01 m/s. Among the types of cellulosic material investigated, only the degree of saccharification for OPFF and CMC was found depended on the impeller diamater.

The feasibility of using hydrolysates from enzymatic saccharification of POME solid and OPFF for acetone-butanol-ethanol (ABE) fermentation by *Clostridium acetobutylicum* P262 was studied using 250 mL modified Schott bottle cultures. The highest solvent produced was obtained in fermentation using hydrolysates treated with activated charcoal. The optimum activated charcoal concentrations required to detoxify the hydrolysates from POME solid and OPFF were 2% (w/w) and 1% (w/w), respectively. Among the carbon sources investigated, the total solvent produced from the POME solid (2.10 g/L) and OPFF (3.24 g/L) hydrolysates were higher than the other carbon sources tested (xylose, cellobiose, and POME solid). Solvent was not produced when CMC and OPFF were used as substrate with butyric acid as the main product, instead.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

SAKARIFIKASI PEPEJAL SISA AIR KILANG KELAPA SAWIT DAN SERABUT BUAH KELAPA SAWIT UNTUK FERMENTASI ACETONE-BUTANOL-ETHANOL

oleh

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Mei 2001

Pengerusi : **Profesor Madya Dr. Arbakariya Ariff**

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Kesan pelbagai rawatan kimia terhadap sakarifikasi pepejal sisa air kilang kelapa sawit (PSAKKS) dan serabut buah kelapa sawit (SBKS) telah disiasat. Antara rawatan kimia yand digunakan ke atas substrak (NaOH 0.5%, NH_3 0.5%, HCl 0.5%, HNO_3 0.5% and EDTA 0.5%), SBKS

yang tertinggi. Tetapi, pencapaian sakarifikasi untuk rawatan kimia PSAKKS tidak menunjukkan perbezaan yang ketara berbanding dengan PSAKKS tanpa rawatan. Autoclave SBKS pada 121°C, 15 psi dengan NaOH selama 5 minit didapati meningkatan darjah hidrolisis sebanyak 46%. Kepekatan optimum NaOH untuk merawat SBKS ialah 2 %. Penambahan dalam hidrolisis PSAKKS dan SBKS telah dikaitkan dengan penambahan kandungan selulose dan pengurangan kandungan hemiselulose serta lignin.

Kesan kepekatan enzim dan substrak terhadap PSAKKS dan SBKS sakarifikasi telah dikaji dengan mengunakan dua jenis enzim, Celluclast (47.4 U/mL Fpase, 66.0 U/mL CmCase and 51.1 U/mL β-glucosidase) and Novozyme 188 (2.79 U/mL Fpase, 10.0 U/mL CmCase and 168 U/mL β-glucosidase). Produksi yang optimum untuk jumlah gula penapaian (9.24 g/L) dan glukosa (4.54 g/L) dari 5% PSAKKS sakarifikasi telah diperolehi dengan menggunakan nisbah Novozyme/Celluclast bernilai 0.4. Manakala sakarifikasi ke atas SBKS dengan penggunaan nisbah N/C yang bernilai 0.25 menghasilkan 32.47 g/L jumlah gula penapaian dan 16.78 g/L glucose. Kesan kepekatan substrak ke atas pencapaian sakarifikasi PSAKKS (2% - 20%) dan SBKS (2% - 6%) telah dijalankan dalam 2 Liter reaktor jenis pengadukan. Jumlah maksimum gula penapaian (12.25 g/L) dan glucose (6.70 g/l) diperolehi apabila 15% (B/I) PSAKKS digunakan. Manakala, jumlah maksimum gula penapaian (30.26 g/L) dan glukosa (16.73 g/L) dihasilkan dari 5% (B/I) SBKS.

Kesan pencampuran ke atas pencapaian sakarifikasi CMC, PSAKKS dan SBKS juga dijalankan dalam 2 Liter reaktor jenis pengadukan dengan menggunakan dua impeller yang berlainan diameter (48 dan 84mm). Dalam sakarifikasi PSAKKS dan SBKS, darjah sakarifikasi bertambah bersamaan dengan penambahan kelajuan hujung impeller (KHI). Tetapi, sakarifikasi CMC hanya bertambah dengan penambahan KHI bawah 2.01 m/s. Di antara ketiga-tiga jenis substrak yang dikaji itu, hanya darjah sakarifikasi untuk SBKS dan CMC didapati bergantung keatas diameter impeller.

Pengajian ke atas fasibiliti penggunaan hidrolisate dari PSAKKS dan SBKS untuk produksi acetone-butanol-ethanol (ABE) oleh *Clostridium acetobutylicum* P262 telah dijalankan dalam 250 mL botol Scott yang diubahsuai. Jumlah pelarut telah diperolehi dari fermentasi ABE yang dirawat dengan arang teraktif. Kepekatan optimum arang teraktif untuk menyahtoksi terhadap hidrolisate dari PSAKKS dan SBKS ialah 2 dan 1 % masing-masing. Di antara sumber karbon yang disiasat, penghasilan jumlah pelarut dari PSAKKS dan SBKS hidrolisate didapati lebih tinggi daripada sumber karbon yang lain (xylose, sellobiose dan PSAKKS). Tetapi, tiada penghasilan pelarut didapati untuk fermentasi ABE yang menggunakan CMC dan SBKS, asid butiric merupakan produk yang utama untuk kedua-dua kes.

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I certify that an Examination Committee met on 21th May 2001 to conduct the final examination of Khaw Teik Seong, on his Master of Science thesis entitled "Saccharification of Palm Oil Mill Effluent Solid and Oil Palm Fruit Fiber to Fermentable Sugars for Acetone-Butanol-Ethanol Fermentation" in accordance with Universiti Putra Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows.

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Master of Science.

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DECLARATION

I hereby declare that the thesis is based on my original work expect for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other at UPM or other institutions.

Name: Khaw Teik Seong

Date: 26/5/2001

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURE

LIST OF ABBREVIATIONS

D	: Impeller Diameter (m)
Ṅ	: Impeller Rotational Speed (rps)
ITP	: Impeller Tip Speed (m/s)
ABE	: Acetone-Butanol-Ethanol
POME	: Palm Oil Mill Effluent
OPFF	: Oil Palm Fruit Fiber
CMC	: Carboxylmethylcellulose
m	: Meter
L	: Litre
mL	: Mililitre
μm	: Micrometer
nm	: Nanometer
rpm	: Rotation per minute
UV	: Ultra Violet
HPLC	: High Performance Liquid Chromatography

CHAPTER 1

INTRODUCTION

The energy requirements for all activities of mankind mainly depended on fossil resources such as petroleum, natural gas and coal. Unfortunately, these fossil resources that deposited and formed over billions of years were limited in stock. Therefore, the exhaustion of these non-renewable fossil fuel stocks in the near future has prompted widespread global efforts for the development of renewable energy resources.

Biomass in the form of photogenic plants is reproduced abundantly year after year with the help of solar energy. According to recent studies, 170×10^9 tonnes of biomass are produced annually as a result of photosynthesis (Table 1). Under the legitimate assumption, biomass consists of about 40% of polysaccharides especially cellulose and starch. The annual production for these photosynthetically produced cellulose and starch are approximately 70 billion tonnes, which can be considered as high yield as compared to finite world reserves of fossil fuel. Ironically, it is only about 3% of there annually renewed biomass are being used. In other word, that is about 66 billion tonnes of these natural raw materials ends up as collectible wastes (Hans, 1993).

In Malaysia, commercial cultivation of oil palm in Malaysia was started in 1917. The pace of the development was slowed to begin with, but picked up rapidly in the 60's

and 70's (Gurmit, 1994). Today, Malaysia is the world's leading producer and exporter of palm oil. The area of palm oil plantation in 1999 was estimated at 3.31 million hectares with the production of about 10.55 million tonnes of palm oil (PORLA, 1999). As a vegetable oil seed crop, oil palm is an efficient converter of solar energy into biomass. Unfortunately, besides being a prolific producer of palm and kernel oil, its also generates a number of lignocellulosic residues and by product such as palm oil mill effluent (POME) and oil palm fruit fiber (OPFF), which are highly polluting. Although the treatment of these lignocellulosic wastes have already been established, the commercially application of these agro-industrial wastes for the production of valuable products is not yet exploited. Thus, an innovative way to treat the POME and OPFF couple with the production of valuable product should be developed.

Table 1: Annual consumption and availability of biomass in the world (in tonnes).

Biomass, annually photosynthesis yeild:	170×10^9
Utilized by or in from of :	
Felling of trees (only major countries)	0.80×10^9
- use for paper	0.15×10^9
- use in chemical applications	0.007×10^9
Cereals (all kinds)	1.45×10^9
Natural fibers (all kinds)	0.022×10^9
Seed products (incl. Oil seeds)	0.18×10^9
- vegetable oils	0.05×10^9
Potatoes	0.37×10^9
Sugar cane and sugar beets	0.58×10^9
Fruits (all kinds)	0.28×10^9
Foodstuffs (of animal origin)	0.28×10^9
Animal feed	0.80×10^9
Total ascertainable utilization	4.969×10^9 (approx. 2.9%)

Source: Hans (1993)

As lignocellulosic materials, POME and OPFF consist of three main chemical components; cellulose, hemicellulose and lignin. The cellulosic portions of lignocellulose are convertible into fermentable sugars, which in turn, can be used to derive polymeric materials, chemical feedstock and solvent (Ghose et al., 1979). The first step in converting lignocellulose to sugars is the saccharifiction process using either acid or enzyme as catalyst. However, the structural properties of lignocelluloses such as lignin-hemicellulose complex and the degree of crystallinity have made the lignocellulose recalcitrant for saccharification. Therefore, various physico-chemical pretreatments have been developed to facilitate the saccharification process over the past few decades. It is accepted that all such pretreaments were added considerably to the overall cost of saccharification. Thus, an economic viable pretreatment process for lignocellulosic material should be investigated to improve production of fermentable sugars through saccharification process.

During the first part of this century, the anaerobic production of acetone-butanol-ethanol (ABE) by solventogenic clostridia was the second largest biotechnological process in the world. This fermentation was initially aimed at the production of acetone for the war industry then the production of butanol for the lacquer industry and later the production of ethanol as biofuel which was mixed together with petrol for automobile industry (Jones and Wood, 1986). After the World War II, petroleum-based production of solvents replaced the biological processes and, as a result, almost all the industrial-scale fermentation facilities have been closed (Durre, 1998). The oil crisis in the 1970s revived interest in ABE fermentation because of the recent